7th Barrande Bioscience Meeting

PRESENTED POSTERS

9 – 11 October 2022 **Olomouc**

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7-phenoxytacrine and its derivatives potently inhibits the GluN1/GluN2B receptor via two independent mechanisms

Abstract

N-methyl-D-aspartate receptors (NMDARs) play a key role in mediating excitatory neurotransmission in the mammalian central nervous system (CNS). NMDARs are heterotetramers containing GluN1, GluN2 and/or GluN3 subunits, giving rise to a wide variety of subunit combinations, each with unique functional and pharmacological properties. Although excitatory glutamatergic neurotransmission via NMDARs is crucial for synaptic plasticity and normal neuronal function, NMDAR overactivity promotes cell death, while NMDAR hypofunction can be a pathogenic trigger for various diseases. Thus, NMDARs are important pharmacological target for the treatment of neurodegenerative diseases, however, new drugs are often associated with a wide range of side-effects. For example, tacrine (THA) was the first FDA approved drug to treat Alzheimer's disease (AD), but was withdrawn from the market because of its hepatotoxicity and other side-effects. However, 7-methoxy derivative of THA (7-MEOTA) has been developed as a pharmacologically similar compound with comparable advantages to THA [1]. We have developed 7-phenoxy derivative of THA (7-PhO-THA) and focused on studying its pharmacological properties and neuroprotective activity, in order to obtain an even better THA analogue than 7-MEOTA [2]. Our electrophysiological data together with a docking analysis showed that 7-PhO-THA potently inhibits the GluN1/GluN2B receptors in a voltage-independent manner via the ifenprodil-binding site, in addition to its voltage-dependent inhibition of both GluN1/GluN2A and GluN1/GluN2B receptors. Moreover, in contrast to 7-MEOTA, 7-PhO-THA effectively inhibits acetylcholinesterase and thus has balanced activity on the cholinergic and glutamatergic systems. Furthermore, 7-PhO-THA demonstrated a potent anti-excitotoxic and neuroprotective effect in the NMDA-induced lesion of the dorsal hippocampus. In addition, using the patch-clamp technique, we tested 30 novel derivatives of 7-PhO-THA and observed glutamatergic current inhibition ranging from 6 to 57% at a concentration of 1 μM. Out of these data, two derivatives (K1958 and K1959) were selected and their IC_{50} values were calculated based on the dose-response relationship (~5-10 μM for GluN1/GluN2A and ~2-3 μM for GluN1/GluN2B). In the further experiments, we will focus on the detailed analysis of their mechanism of action which I will present on my poster. Taking together, our results suggest that 7-PhO-THA is a promising compound which equally act through cholinergic and glutamatergic systems with a low risk of psychotomimetic side-effects, and consequently, novel 7-PhO-THA derivatives can be a promising target for investigation as a potential treatment for neurodegenerative diseases including AD.

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2. Jan Kretschmer, *Institute of Organic Chemistry and Biochemistry of the CAS, Flemingovo náměstí 542/2, 160 00 Prague 6, Czech Republic.* [2] *Department of Organic Chemistry, Faculty of Science, Charles University in Prague, Hlavova 2030/8, 128 43 Prague 2, Czech Republic.*

Synthesis of paramagnetically encoded molecules with DO3A-Hyp building blocks

Abstract

Macrocyclic chelators of the DOTA type form stable complexes with lanthanide ions that find various biomedical applications, such as contrast agents for magnetic resonance imaging or targeted radiotherapeutics. Here we report a new family of synthetic building blocks, named DO3A-Hyp, that combine the properties of macrocyclic chelators with the amino acid hydroxyproline. The amino acid functionality provides the possibility to link multiple (different) lanthanide ions within a single peptide chain. The design of DO3A-Hyp allows to achieve total control over the positions of the metal ions. We report several synthetic strategies leading to such multi-metallic compounds. The sequence of incorporated metal ions can bear digital information, similarly to nucleotides in DNA. However, owing to the paramagnetic properties of lanthanides, the sequence is readable by NMR spectroscopy. The reported molecules can potentially serve as programmable digital tags for labeling of micro/nano objects compatible with in-vivo applications.

3. Kevin Kotalík, *Institute of Macromolecular Chemistry, Czech Academy of Sciences, Heyrovského nám. 2, 162 06 Prague 6, Czech Republic*

Water-soluble polymer conjugates with 5-aminolevulinic acid intended for photodynamic therapy

Abstract

Photodynamic therapy (PDT) is a promising strategy in cancer treatment. Recently, 5-aminolevulinic acid (5-ALA), a precursor of protoporphyrin IX, has been studied intensively as a prodrug for PDT. However, this approach still lacks tumor selectivity and thus can cause skin photosensitivity; there is also a need to repeat the process due to 5-ALA rapid clearance. Having the aforementioned in mind, we developed new nanotherapeutics based on poly(*N*-2-(hydroxypropyl)methacrylamide) – PHPMA copolymers with 5-ALA conjugated via a pH sensitive hydrazone bond. These water-soluble polymer conjugates are expected to circulate for prolonged time in the body and to accumulate in tumor tissue at a higher rate than free 5-ALA due to the enhanced permeability and retention (EPR) effect, and therefore increase the efficacy of PDT. Herein, we present the controlled synthesis and characterization of these nanomaterials as well as *in vitro* cytotoxicity evaluation with or without irradiation of cells treated with these polymer conjugates. Moreover, the results of preliminary evaluation of treatment efficacy are presented.
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4. Alžběta Turnovská, *Institute of Macromolecular Chemistry, Czech Academy of Sciences, Prague, Czech Republic*

Polymer conjugates with porphyrins for photodynamic therapy and tumour imaging

Abstract

Photodynamic therapy (PDT) uses a light-sensitive drug known as photosensitizer (PS) in combination with light irradiation of appropriate wavelength. Light-activated form of the drug reacts with the oxygen present in tumour tissue leading to the formation of reactive oxygen species (ROS), i.e. singlet oxygen ($^1O_2$), that induce tumour cells death.[1] Biocompatible, water-soluble and non-toxic nanomaterials such as *N*-(2-hydroxypropyl)methacrylamide (HPMA) copolymers can be used to enhance the drug accumulation in solid tumours thanks to the so-called Enhanced Permeability and Retention effect (EPR), thus improving the therapeutic outcome.[2] This study presents the synthesis and physico-chemical characterisation of HPMA-based copolymers conjugated with Palladium(II)-tetraphenyl-tetrabenzoporphyrin (PdTPTBP) or pyropheophorbide-a (PyF) as possible tumour-targeted theranostics. HPMA-based carriers were synthesised by the controlled radical reversible addition-fragmentation chain transfer (RAFT) polymerisation. PdTPTBP derivatives were attached to the polymer backbone via either a stable amide bond or a pH-sensitive hydrazone bond, while PyF derivative was covalently attached via an amide bond. The use of HPMA backbone allowed the solubilization of both PdTPTBP and PyF. Their respective polymer theranostics carried from 2 to 7 wt.% of photosensitizer and the hydrophobic character of these compounds resulted in the self-assembly of these systems into micelles with hydrodynamic diameters ranging from 20 to 40 nm. The cytotoxicity of conjugates with PyF was remarkably increased when light was irradiated and they showed high tumour-targeted accumulation. These materials exhibited a remarkable tumour imaging with high sensitivity and low background as well as a potent antitumor PDT effect in different solid tumour models. Therefore HPMA-based theranostics are promising candidates for tumour-targeted photodynamic therapy and diagnosis.

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5. Dinesh Dhumal, *Centre Interdisciplinaire de Nanoscience de Marseille (CINaM), UMR 7325 CNRS, Aix Marseille Uni., Marseille, France.*

Dynamic self-assembling supramolecular dendrimer nanosystems as potent antibacterial candidates against drug-resistant bacteria and biofilm

Abstract

The alarming increase and prevailing nature of antibiotic resistance urge for new antibacterial agents, preferably those differing substantially from conventional antibiotics [1]. Amphiphilic antibacterial agents are particularly appealing to tackle this crisis because they can mimic the antibacterial features of natural antimicrobial peptides and antibacterial detergents [2]. Additionally, self-assembled supramolecular nanostructures of these amphiphilic agents can contribute towards the antibacterial activity via cooperative and multivalent interaction. [3] In this perspective, we have developed amphiphilic dendrimers and explored their potential as antibacterial candidates. We report here novel antibacterial candidates based on self-assembling amphiphilic dendrimers composed of a hydrophobic alkyl chain and a hydrophilic poly(amidoamine) dendron bearing different terminal functionalities [4-5]. Remarkably, the dendrimer with amine terminals exhibited strong antibacterial activity against both Gram-positive and Gram-negative as well as drug-resistant bacteria and prevented biofilm formation [4]. In addition, the amphiphilic dendrimer is able to form nanomicelles and co-deliver antibacterial agents. Our study presents a novel concept for generating potent antibacterial candidates and offers a new perspective for combatting antibacterial resistance.

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6. **Petra Čechová,** *Regional Center of Advanced Technologies and Materials, The Czech Advanced Technology and Research Institute (CATRIN), Palacký University Olomouc, Šlechtitelů 27, 779 00*
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Interactions of Ionizable and Membrane Lipids

Abstract

Lipid nanoparticles (LNPs) as the carrier systems of pharmacologically active compounds have entered the spotlight during the Covid19 pandemics in the mRNA containing vaccines. However, the technology of nanoparticle drug delivery has been also studied as a tool for cancer or rare disease therapy. The advantage of LNPs comprised of ionizable lipids (ILs) is in the tunability of the IL properties to achieve optimal properties and support the targeted delivery into the organ of choice.
On its way from entering the body to releasing its cargo in the target cell, the LNP has to move across the cell membrane - either by interacting with it directly, or via endocytosis. During this process, it inevitably encounters and interacts with lipids naturally occurring in these membranes.
This is however challenging to study experimentally. To understand the molecular details of these interactions, a series of molecular dynamics simulation was created, to observe the effect of each natural membrane lipid separately. In a combination of free and biased simulations, the merging of the LNP with membrane lipids with different headgroups does indeed differ, largely based on the charge of the headgroup. As the ionizability is a key feature of the ILs, these findings are both in line with expectation, and provide a molecular resolution to LNP-membrane fusion.

7. **Klára Gajdošová ,** *Department of Physical Chemistry, Faculty of Science, Palacký University Olomouc, 17. listopadu 12, 771 46 Olomouc, Czech republic*

Graphene-based Biosensors for Multimodal Detection of Breast Cancer Biomarkers

Around 15-20% of all diagnosed breast cancers belong to a group called triple negative breast cancer (TNBC). TNBC contains 6 molecular subtypes that are all very aggressive and have poor survival rate. They are collectively characterized by negative findings of the progesterone receptor, estrogen receptor, and the HER2 gene. Currently, there is no routinely used biomarker for this group of breast cancer.

Protein C receptor ($Procr^+$), a cell surface protein, was recently correlated to cancer stem cells (CSCs) in about half of triple negative breast cancer cases. Inhibition of $Procr^+$ caused reduction in the number of CSCs, stopped the growth of the tumour, and was beneficial against swift tumour recurrence.

In this work, we proposed graphene-based biosensors for protein C receptor detection. Synthesis of such materials starts with fluorographene chemistry that allows immobilization of amino acids – in this case arginine, that can be further functionalized with specific antibodies. This platform is later tested using electrochemical impedance spectroscopy and Raman spectroscopy.

Firstly, we attempt to apply graphene-arginine as a signal enhancing platform for graphene enhanced Raman spectroscopy (GERS). Graphene enhancement can reach up to two orders, however it works only with a monolayer of graphene flakes, ideally without defects. Testing of the enhancing capabilities of our synthesised material is done with reporter molecule rhodamine 6G.

For electrochemical impedance spectroscopy, the derivative is further functionalized with a PROCR specific antibody. This material is tested for varying concentrations of PROCR in 0.1M MES buffer, and its specificity against rabbit antigen.

8. Mohammed Karin Sebanne, *Team 3BIO (Biovectorisation, Bioconjugation and Biomaterials), UMR7199 Design and Application of Bioactive Molecules, University of Strasbourg, 74 Rte du Rhin, 67400 Illkirch- Graffenstaden*

Development of an antitumor vaccine approach based on the delivery of messenger RNA

Cancer immunotherapy is defined as the ability to mobilize the host's immune system to kill cancer cells. It has recently taken a central role within mainstream oncology with the use of immune checkpoint inhibitors and has shown unprecedented clinical responses in patients. Despite this success, broad immunotherapy can result in severe adverse effects such as autoimmunity, highlighting the need for new therapies. In the last decades, therapeutic cancer vaccines have proven to be able to induce strong immune responses with little-to-no adverse effects [1]. Capable of eliciting exceptionally strong immune responses, RNA has emerged as an attractive vaccine platform for cancer therapy [2]. Thus, we propose to develop innovative mRNA-based vaccine formulations that will allow the establishment of an effective anti-tumor immune response. Current mRNA vaccines are based on cationic lipid formulations which have been shown to induce Reactive Oxygen Species and cell death on immune cells [3]. They also contain small amounts of PEG that has been shown to be immunogenic, impairing use of the same nanoparticles for future vaccines [4]. Thus, developing suitable next-generation mRNA-based nanoparticles for vaccination is a major challenge

As a part of our work, we designed pH-triggered cell penetrating peptides (CPPs) derived from viral fusogenic peptides [5]. We investigate the ability of those CPPs to efficiently transfect mRNA into dendritic cells (DCs), the antigen presenting cells (APCs) which are the initiators of the immune response. By using mRNA coding for the reporter gene green fluorescent protein (GFP), we observe a strong transfection efficiency of various APCs (murine macrophages RAW264.7 and mouse DC2.4) - and lower cytotoxicity as compared to the commercial lipidic transfection agent Lipofectamine. As RNA is also a danger signal to the immune system, we confirm the ability of our constructs to induce the activation of APCs by analyzing the overexpression of activation marker CD40 on the surface of those cells by flow cytometry.

Henceforward, we aim to ensure the ability of our formulations to induce the presentation of the mRNA encoded antigen by the DCs and their ability to induce the activation of effector immune cells by co-culture with T-cells (responsible for the killing of cancer cells). Our molecules could thus represent an easy-to-formulate platform for mRNA vaccination that could be very interesting for cancer therapy.

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